

No Association Between His 447 His Polymorphism of PPAR γ Gene and Osteoporosis in Iranian Postmenopausal Women

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Abstract

Background: Osteoporosis is frequently observed in postmenopausal women. Evidence indicates that the role of genetic factors is significant in osteoporosis. This study aimed to inspect the potential association between His 447 His polymorphism of PPAR γ gene and bone mineral density (BMD) and serum lipid profiles in postmenopausal women suffering from osteoporosis compared to the healthy controls.

Methods: The study was conducted on 224 postmenopausal women including 107 osteoporosis patients (age: 61.03 ± 4.54 years) and 117 healthy controls (age: 55.73 ± 2.08 years). Blood samples were analyzed for polymorphism of PPAR γ gene using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Multivariate analysis was used to investigate the relationship between the risk of osteoporosis and PPAR γ gene polymorphisms.

Results: Serum low density lipoprotein (LDL) and total cholesterol (TC) levels were significantly different between the osteoporosis group and the control group ($P < 0.001$). After adjustment for factors such as TC and LDL, logistic regression analysis showed that the risk of osteoporosis was not significantly higher in carriers of homozygous wild-type genotype than carriers of the rare alleles ($P = 0.99$, odds ratio = 0.997, 95% confidence interval = 0.52 - 1.91).

Conclusions: This study suggests that His 447 His polymorphism of PPAR γ gene does not play the role of an independent factor in BMD and osteoporosis in postmenopausal women.

Keywords: Osteoporosis, Postmenopausal Women, Bone Mineral Density, Ppar γ , His 447 His Gene Polymorphism

1. Background

Osteoporosis is the most common metabolic bone disease that can lead to increase of fragility of the bone tissue (1, 2). The disease is more common in women than men because they have a smaller bone mass and, during post menopause, they produce less sex steroid hormones, which decreases the body's ability to retain calcium in the bones (2). Previous studies show that osteoporosis affects up to 50% of Iranian men and women over 50 years (3). Osteoporosis is characterized by reduced bone mineral density (BMD) and loss of bone microstructure (4). The best way to measure BMD is to use dual energy X-ray absorptiometry (DXA) (4). In previous years, studies have proposed genetic factors as a major cause of BMD regulation (2, 5). Peroxisome proliferator-activated receptor (PPAR)- γ belongs to the nuclear hormone receptor family, which is predominantly expressed in the adipose tissue, and also plays an important role in the bone micro environment (6). In addition, PPAR- γ activation seems to have regulatory effects on bone metabolism through regulating their differentiation or activation into osteoclasts (7-9). How-

ever, evidence on whether the activation of PPAR- γ may affect bone metabolism through the activation or inhibition or cell lineages is not enough for conclusion. On the other hand, PPAR γ gene is located on short arm chromosome 3 (3p25) in human and is composed of 9 exons (10). Several single nucleotide polymorphisms (SNP) have been detected in PPAR γ in human. The His 447 His polymorphism (rs3856806) is known as C \rightarrow T substitution in exon 6. The His 447 His polymorphism is the most frequently found genetic variant of PPAR γ that has also been known as gender-specific genetic modulator of metabolic homeostasis (11). The less common T allele of the silent His 447 His polymorphism has been associated with low PPAR activity and decreased ovarian androgen biosynthesis (12). Ogawa et al. showed that there is a significant association between His 447 His polymorphism of PPAR γ gene and BMD in postmenopausal Japanese women (13). Also, a previous polymorphism study performed among Korean women showed a significant relationship between this polymorphism and serum osteoprotegerin (OPG) level, as a key inhibitor of osteoclastogenesis (14). Since genetic variations

could be of potential relevance to osteoporosis in postmenopausal women, for the first time, we investigated in this study the influence of PPAR γ gene polymorphism on osteoporosis in Iranian postmenopausal women.

2. Methods

2.1. Subjects

This case-control study was conducted on 224 postmenopausal women who referred to the Bu-Ali hospital of Qazvin, between January 2013 and February 2015. The study was approved by the ethics committee of Qazvin University of Medical Sciences, Qazvin, Iran. A total of 107 women with osteoporosis (case group) with the mean age of 61.03 ± 4.54 years and 117 healthy postmenopausal women (control group) with the mean age of 55.73 ± 2.08 years were selected. The women in the two groups were carefully matched for BMI. None of the participants were on special diet, none were cigarette smokers, and none were alcohol consumers. Women were excluded from the study if they had self-reported fracture history, known dyslipidemia, premature menopause, and systemic disease such as thyroid dysfunction, diabetes mellitus, parathyroid disease, liver disease, and renal failure that might affect bone metabolism or trace elements status. None of the subjects had received hormone replacement therapy and anabolic steroids, bisphosphonates, calcitonin, lipid-lowering drugs, calcium and vitamin D supplements during the six months preceding the onset of the study. All of the participants completed a questionnaire including demographic characteristic such as age, BMI, nutritional status, previous fracture, and use of medicine.

2.2. BMD Measurements

BMD (g/cm²) was measured in the femur neck and lumbar spine 1-4 (L1-4) by DXA QDR

2000 (Hologic, Bedford, MA, USA). The coefficient of variability values of DXA measurements was 1.0% for lumbar spine vertebra and 1.2% for femoral neck (14). Subjects with spine or femur neck BMD 2.5 standard deviations (SD) below a reference range (T score ≤ -2.5) were accepted as having postmenopausal osteoporosis (15). The control group (non-postmenopausal osteoporosis) consisted of women with a T score ≥ -1.5 on these sites.

2.3. Lipid Profile and Serum Parameters Analysis

Total cholesterol (TC), HDL-C, and triglyceride (TG) were determined by standard enzymatic methods. LDL-C was calculated by Friedewald formula: $LDL-C = TC - (HDL-C + TG/5)$ (16). Serum total calcium (normal range 8.5 - 10.5 mg/dL) was measured by using a colorimetric assay

while serum vitamin D was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Alpco Diagnostics, Windham, United States).

2.4. Genotyping

Genetic analyses were performed on genomic DNA isolated from peripheral blood leukocytes by standard methods. A 181 bp sequence of the PPAR γ gene was amplified by polymerase chain reaction (PCR) in a DNA thermal cycle (ABI, Veriti, USA) by using oligonucleotide primers 5'-CCAGAAAATGACAGACCTCAGACA-3' F, and 5'-CAGAATAGTGCAACTGGAAGAAGG-3' R.

The PCR condition was 95°C for 5 minutes, 35°C cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 7 minutes (17). Restriction of the PCR product with the PmlI enzyme generates fragment of 142/39 bp in rare homozygotes, 181/142/39 bp in heterozygotes, and 181 bp in common homozygotes. Samples were electrophoresed on 3% agarose gel and stained with ethidium bromide.

2.5. Statistical Analysis

Values were presented as mean \pm SD, and statistical significance was defined as P values less than 0.05. Statistically significant differences in mean measurements between different parameters were performed using t-test. Correlation analysis between variables was performed by calculating Pearson's correlation coefficients. All analyses were carried out using statistical software package, SPSS, V 11.0, for windows (Chicago, IL, USA).

3. Results

Demographic data and clinical characteristics are shown in Table 1. Women with postmenopausal osteoporosis had significantly higher age ($P < 0.001$) but similar BMI compared to non-osteoporotic controls. Comparison of lipid profile in patients and controls shows that the TC levels were higher in the osteoporosis group than the control group (218.15 ± 48.94 mg/dL vs. 195.9 ± 31.9 mg/dL, $P < 0.001$). Moreover, the LDL level of the osteoporosis group was higher than that of the control group (122.15 ± 22.16 mg/dL vs. 104.2 ± 31.9 mg/dL, $P < 0.001$). The TG levels were not significantly different between the groups (osteoporosis group: 105.26 ± 18.25 mg/dL, control group: 116.3 ± 45.5 mg/dL, $P = 0.02$). Similarly, the HDL levels were not significantly different between the groups (osteoporosis group: 46.5 ± 6.9 mg/dL, control group: 49.3 ± 8.6 mg/dL, $P = 0.01$) (Table 1).

The genotype distribution of the His 447 His polymorphism of PPAR γ gene in both groups was in the Hardy-Weinberg equilibrium (both $P > 0.05$). The distribution

Table 1. Metabolic Parameters of Patients with Osteoporosis Versus Controls^a

	Controls (n = 117)	Patients (n = 107)	P Value
Age (years)	55.73 ± 2.08	61.03 ± 4.54	< 0.001
BMI (kg/m ²)	27.94 ± 3.2	28.13 ± 2.58	0.64
BMD-F	-0.25 ± 0.83	-2.29 ± 0.82	< 0.001
BMD-S	-0.45 ± 0.72	-2.79 ± 0.66	< 0.001
Ca (mg/dL)	9.4 ± 0.48	9.44 ± 0.54	0.69
VitD (nmol/L)	35.2 ± 10.9	37.2 ± 10.3	0.169
Triglyceride (mg/dL)	116.3 ± 45.5	105.26 ± 18.25	0.02
Cholesterol (mg/dL)	195.9 ± 31.9	218.15 ± 48.94	< 0.001
LDL-C (mg/dL)	104.2 ± 31.9	122.15 ± 22.16	< 0.001
HDL-C (mg/dL)	49.3 ± 8.6	46.5 ± 6.9	0.01

^a Values indicate Mean ± Standard deviation.

of genotypes was not different between the osteoporosis group and the control group (79.4% CC, 16.8% CT, 3.7% TT vs. 79.5%, 18.8%, 1.7%, respectively, $P = 0.6$) (Table 2).

The risk of osteoporosis in different genetic groups was calculated by logistic regression analysis (Table 3).

This analysis showed no statistically significant difference between the groups in the risk of endometriosis ($P = 0.99$, odds ratio [OR] = 0.995, 95% confidence interval [CI] = 0.47 - 2.09) after adjustment for TC and LDL-C. ANOVA test also showed no statistically significant difference in metabolic parameters in the study women (Table 4). There was no difference even when the control and osteoporosis groups were analyzed separately.

4. Discussion

Peroxisome proliferator-activated receptor gamma (PPAR- γ) is an essential cellular factor for the control over a number of physiological processes and pathogenesis of various diseases. PPAR- γ is an important transcription factor in adipocyte differentiation that seems to have an important role in bone metabolism. Recent studies have shown that PPAR- γ not only suppresses osteoblast formation, but also plays a vital role in osteoclastogenesis and bone resorption with the help of PPAR- γ coactivator 1 β (PGC1 β) and estrogen-related receptor alpha (ERR α) (18). In addition, numerous studies have discussed the role of various PPAR- γ gene polymorphisms on lipid metabolism as well as bone turnover (13, 14, 17, 19, 20). Therefore, the aim of this study was to evaluate the effects of polymorphisms in the PPAR- γ gene on bone mineral density (BMD) and lipid profile of postmenopausal women with or without osteoporosis. Our findings suggested no association

of different types of PPAR- γ gene polymorphism with BMD. This finding is consistent with those of Yu et al. study conducted among Chinese women (13).

However, the results of many studies indicate the relationship between PPAR- γ gene polymorphisms and BMD (20). Surveys conducted in Korea and Japan have found that C161T (His 447His) polymorphism is associated with decreased levels of OPG and BMD, particularly prominent among postmenopausal women (13, 14, 19). Rhee et al. have also demonstrated that those with Pro2Ala polymorphisms in PPAR- γ gene have reduced OPG levels in their serum (17). This discrepancy could be due to different ethnic background and sample size of this study with other surveys, as Rooki et al. evaluation of C161T and Pro12Ala polymorphisms of PPAR- γ gene among Iranian population has shown that the prevalence of such disturbances is significantly different from those of other investigated populations, including Korean population (21).

In addition, the results of this study supported that bone density levels are inversely associated with serum levels of LDL and total cholesterol, as postmenopausal women with osteoporosis showed significant differences in LDL and total cholesterol values. Other conducted studies also discovered evidence in favor of the correlation between BMD and serum lipid levels among postmenopausal women (22). However, the serum lipid profile showed no significant difference in all the 3 studied polymorphisms of PPAR- γ gene. Zafarmand et al. have also demonstrated that the PPAR- γ gene polymorphisms in healthy Dutch women do not have a major impact on the average of lipid profile (23).

Generally, the results of this study indicated that there is no difference in the prevalence of PPAR- γ gene poly-

Table 2. Genotype Distributions in Patients with Osteoporosis Versus Controls^a

Genotype	Control	Patients	Total
CC	93 (79.5%)	85 (79.4%)	178 (79.5%)
CT	22 (18.8%)	18 (16.8%)	40 (17.9%)
TT	2 (1.7%)	4 (3.7%)	6 (2.7%)
	117	107	224

^a P Value = 0.60.**Table 3.** Logistic Regression Analysis of Alleles with Respect to the Presence of Osteoporosis as Dependent Variable^a

SNP Allele Compared to Non-Carriers	Univariate			Multivariate ^b		
	OR	95% CI	P	OR	95% CI	P
His 447 His	0.997	0.52 - 1.91	0.99	0.995	0.47 - 2.09	0.99

^a Values are 95% confidence interval (CI), odds ratio (OR). P value: chi-square test.^b Adjusted for TC and LDL-C.**Table 4.** Metabolic Parameters According to the Genotypes

	CC (n = 178)	CT (n = 40)	TT (n = 6)	P Value
Age (years)	58.2 ± 4.2	58.4 ± 5.1	56.8 ± 3.3	0.70
BMI (kg/m ²)	27.9 ± 2.9	28.4 ± 2.7	28.3 ± 1.3	0.52
BMD-F	-1.19 ± 1.3	-1.2 ± 1.1	-2.1 ± 2.1	0.24
BMD-S	-1.5 ± 1.3	-1.6 ± 1.2	-1.7 ± 1.8	0.91
Ca (mg/dL)	9.4 ± 0.7	9.4 ± 0.5	9.4 ± 0.4	0.95
Vit D (nmol/L)	36 ± 1	35.2 ± 9.5	45 ± 9.1	0.11
Triglyceride (mg/dL)	111.6 ± 36	110.4 ± 32	96.6 ± 8	0.59
Cholesterol (mg/dL)	207.5 ± 44	199.2 ± 35	224.3 ± 28	0.31
LDL-C (mg/dL)	113 ± 20	111 ± 17	116 ± 16	0.79
HDL-C (mg/dL)	48 ± 8	47 ± 8	47 ± 8	0.88

morphisms among postmenopausal women with or without osteoporosis. The study also showed no significant changes in LDL and total cholesterol levels in individuals with different polymorphisms of PPAR- γ gene. To our knowledge, this is the first study that evaluated the polymorphisms of PPAR- γ gene in postmenopausal women with osteoporosis. Lack of estrogen level assessment as an intervening variable in bone and lipids metabolisms was the limitation of our study.

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Footnote

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